

NUCLEIC ACID ENCODING SPINOCEREBELLAR ATAXIA-2  
AND PRODUCTS RELATED THERETO

BACKGROUND OF THE INVENTION

Disorders of the cerebellum and its connections are a major cause of neurologic morbidity and mortality. One of the cardinal features of lesions in these pathways is ataxia or incoordination of movements and gait. Although some of the lesions have obvious etiologies such as trauma, strokes or tumors, the etiology of many ataxias has remained difficult to define and is due to metabolic deficiencies, remote effects of cancer or genetic causes. Hereditary spinocerebellar degenerations have a prevalence of 7 - 20 cases per 100,000 (Filla et al., *J. of Neurology* 239(6):351-353 (1992); Polo et al., *Brain* 114 (pt2):855-866 (1991)) which equals the estimates for the prevalence of multiple sclerosis in the United States. Based on clinical analysis and genetic inheritance patterns several forms of ataxias are now recognized. Among the genetic causes of ataxic disorders, the autosomal dominant spinocerebellar ataxias (SCAs) have been the most difficult to classify and until recently no clues to their cause existed.

The SCAs are progressive degenerative neurological diseases of the nervous system characterized by a progressive degeneration of neurons of the cerebellar cortex. Degeneration is also seen in the deep cerebellar nuclei, brain stem, and spinal cord. Clinically, affected individuals suffer from severe ataxia and dysarthria, as well as from variable degrees of motor disturbance and neuropathy. The disease usually results in complete disability and eventually in death 10 to 30 years after onset of symptoms. The genes for SCA types 1 and 3 have been identified. Both contain CAG DNA repeats that cause the disease when expanded. However, little is known how CAG repeat expansion and consequent elongation of

polyglutamine tracts translate into neurodegeneration. The identification of the SCA2 gene would provide the opportunity to study this phenomenon in a new protein system.

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The significance of identifying ataxia genes goes beyond improved diagnosis for individuals, the possibility of prenatal/presymptomatic diagnosis or better classification of ataxias. Most of the genes associated with repeat expansions in the coding region including the genes for SCA1 and SCA3 are genes that show no homology to known genes. Thus, isolation of these genes will likely point to pathways leading to late-onset neurodegeneration that are novel and may have importance for other neurodegenerative diseases.

For example, it has been suggested that CAG expansion may result in increased transglutamination of proteins, a process that has also been implicated in Alzheimer's disease. The ataxias in particular offer the unique opportunity to study how different genes may either independently or through conjoined action in the same pathway produce relatively similar phenotypes in humans. Therefore, it may be possible to examine the interaction of these genes on age of onset and phenotype, and explain that part of phenotypic variability that is not explained by determining repeat expansion in the mutant allele. Cosmids and YACs have been the main tools for generating contig maps of chromosomal regions and the entire genome, respectively. Recently, novel cloning vectors (reviewed in Ioannou et al., *Nat. Genet.* 6:84-89 (1994)) have been developed that may be more stable than cosmids, while being considerable larger.

Several systems of classification have been proposed for the SCAs based on pathological, clinical or genetic criteria. However, these attempts have been

hampered by the extreme variability of disease onset and clinical features within and between families. Among the dominant ataxias only Machado-Joseph disease (MJD) has been clinically defined as a separate disease based on the prominence of basal ganglia involvement. However, since phenotypic variability is remarkable in MJD pedigrees, the assignment of individual cases or small families to this category is difficult. Indeed, after identification of the MJD locus (SCA3) it has become apparent that families with a phenotype not typical of MJD, but resembling SCAs are linked to the same locus as SCA3 families.

The advent of genetic linkage analysis provided a novel means to approach classification of the SCAs. Since the late 70's it was recognized that some SCA pedigrees appeared to show linkage to the HLA locus on CHR6, while others did not. Later this locus, now called SCA1, was further defined using RFLP and microsatellite markers and was mapped centromeric to the HLA locus. After the establishment of flanking markers for the SCA1 gene it became rapidly apparent that many- if not the majority- of SCA families did not show linkage to the SCA1 locus. Recently, a second SCA locus was identified on CHR12 using a large pedigree of Cuban descent (Gispert et al., Nat. Genet. 4:295-299 (1993)) and in a pedigree of Southern Italian origin (Pulst et al., Nat. Genet. 5:8-10 (1993)). At the same time a third locus for Machado-Joseph disease and other pedigrees with an SCA phenotype was identified on CHR14 (Takiyama et al, Nat. Genet. 4:300-304 (1993)). Recently, SCA4 was mapped to CHR16 and SCA5 to CHR11 (Ranum et al., Nat. Genet. 8:N3:280-284 (1994)).

Two of the SCA genes have been identified, one by a positional cloning approach, the other by a cDNA based approach. The SCA1 gene was identified by screening a cosmid contig covering the region between the two flanking

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markers D6S274 and D6S89 for cosmids containing CAG repeats. A CAG repeat was isolated, and shown to be expanded in affected individuals (Orr et al., *Nat. Genet.* 4:221-226 (1993); see Table 1). The number of CAG repeats are inversely correlated with the age of onset. Recently, the complete coding sequence for the SCA1 gene has been determined. The gene does not appear to be homologous to other known genes. Despite the tissue specific effects of the mutation, SCA1 transcripts are ubiquitously expressed. By RT-PCR analysis, normal and mutated transcripts are found in tissues indicating that repeat expansion does not interfere with transcription.

The SCA3 or MJD gene was identified after several CAG containing cDNA clones had been isolated from a brain cDNA library (Kawaguchi et al., *Nat. Genet.* 8:221-227 (1994)). One of these mapped to CHR 14q32.1, the region previously identified by genetic linkage analysis to contain the SCA3 gene. The CAG repeat was expanded in affected individuals, but appears to show greater meiotic stability than other CAG repeats. The SCA3 gene has no homology to other known genes or motif structures, but related sequences were identified on CHR 8q23, 14q21, and Xp22.1.

Although not an SCA gene in the strict sense, CAG expansion in the gene causing dentatorubral-pallidoluysian atrophy (DRPLA) may also lead to degeneration of cerebellar neurons. This gene was identified by searching published brain cDNA sequences for the presence of CAG repeats. A cDNA mapped to CHR12p was found to harbor a CAG repeat which was expanded in DRPLA patients (Koide et al., *Nat. Genet.* 6:9-13 (1994); Nagafuchi et al., *Nat. Genet.* 6:14-18 (1994)). The gene which has no known homologies is ubiquitously expressed. SCA families linked to markers on CHR 12 have been described in several ethnic backgrounds.

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The phenomenon of unstable DNA repeats raises many fascinating issues. For example, in 1991, La Spada et al. identified a polymorphic CAG repeat in the androgen receptor gene on the X chromosome that was greatly expanded in individuals with spinobulbar muscular atrophy (SBMA, Kennedy syndrome). In short succession, a total of ten diseases were found to be caused by trinucleotide repeat (TNR) expansion (Table 1). Although several unifying concepts emerge from the comparison of diseases caused by TNR expansion, important differences can be recognized as well.

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TABLE 1:

Characteristics of diseases caused by TNR expansion

	Disease	Type of of repeat	Location of of repeat	Number of repeats in normal alleles in disease alleles	
5	-----	-----	-----	-----	-----
	Fragile X syndrome	CGG	5' untr.	5 - 54	200 - 200
	FRAXE	GCC	unknown	6 - 25	200 - 80
	FRAXF	GCC	unknown	6 - 29	300 - 500
	FRA16A	GCC	unknown	16 - 49	1000 - 20000
10	Myotonic dystrophy	CTG	3' untr.	5 - 35	100 - 200
	SBMA	CAG	coding	11 - 31	40 - 62
	Huntington disease	CAG	coding	15 - 38	38 - 120
	CA 1	CAG	coding	25 - 36	43 - 81
	DRPLA	CAG	coding	7 - 26	49 - 75
15	MJD (SCA3)	CAG	coding	13 - 36	68 - 79

TNR expansion may be a common form of human  
 mutagenesis. Especially if expansion is not restricted  
 to pure CAG and CCG repeats, the number of genes  
 predisposed to expansion may be quite large. Three  
 diseases with cerebellar degeneration, SCA1, DRPLA, and  
 SCA3 are caused by expansion of a CAG repeat. In these  
 diseases clear evidence of anticipation was lacking,  
 although very early onset cases in some families had  
 raised this question. However, as described in Pulst et  
 al. (1993) strong evidence for anticipation was  
 identified in the FS pedigree with SCA2. Thus, there is  
 a need in the art to identify the location and nucleic  
 acid structure of the SCA2 gene.

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic  
 acids encoding the human SCA2 protein and isolated  
 proteins encoded thereby. Further provided are vectors  
 containing invention nucleic acids, probes that hybridize  
 thereto, host cells transformed therewith, antisense  
 oligonucleotides thereto and compositions containing,  
 antibodies that specifically bind to invention  
 polypeptides and compositions containing, as well as  
 transgenic non-human mammals that express the invention  
 protein. In addition, methods for diagnosing

spinocerebellar Ataxia Type 2, or a predisposition thereto, are provided.

#### BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows a physical map of the SCA2 region. The location of D12S1328 centromeric and D12S1329 telomeric of the contig are indicated. As indicated by double forward slashes, the map is not drawn to scale between D12S1328 and P46F2t7, and between B78E14t7 and D12S1329. YAC, PAC and BAC clones are prefixed with 'Y', 'P', and 'B' respectively. Clones positive for a specific STS by PCR analysis are indicated by vertical lines. Solid arrows indicate end-STSS from the clone under the symbol. Sizes of all clones are shown to scale. The chimeric part of YAC clone 856\_h\_2 (1,100 kb) is indicated by a dashed arrow. Interstitial deletions in YACs or PACs are indicated by thin lines in brackets. The extent of the deletion in YAC Y638 .e.7 is not precisely known.

Figure 2 shows the nucleic acid sequence (SEQ ID NO:1) of plasmid PL65I22B for genomic DNA encoding the expansion of the CAG repeat in individuals with SCA2. Nucleotides 1 - 499 of Figure 2 correspond to cDNA nucleotides 392 - 890 of Figure 6 (SEQ ID NO:2). The locations of primers SCA2-A and SCA2-B are indicated by arrows. The location of a predicted splice site is indicated by a vertical arrow between nucleotides 499 and 500 (also compare with Figure 6).

Figure 3 shows an analysis of the SCA2 CAG repeat by polyacrylamide electrophoresis. A common allele of 22 repeats and a less frequent allele of 23 repeats (samples 14 and 15) are seen in normal individuals. SCA2 patients with extended alleles form 37

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to 52 repeats are shown. SCA2 patients derive from two pedigrees with CHR 12 linked dominant ataxia. The pedigree structures are shown at the top. Genomic DNAs were amplified with primers SCA2-A and SCA2-B and separated in a 6% polyacrylamide gel. Primer SCA2-A was end-labeled. As a size standard, single stranded M13mp18 control DNA was sequenced with sequencing primer "-40" provided by USB (United States Biochem.).

Figure 4 shows a Scattergram indicating that CAG repeat length and age-of-onset of disease in 33 SCA2 patients are inversely correlated.

Figure 5 shows four cDNA clones as a schematic of the composite SCA2 cDNA sequence. The thick line corresponds to coding sequence, the thin line to untranslated regions. The location of the CAG repeat is indicated by a hatched box. In clone S2, the repeat was not a CAG, but a CTG repeat followed by 12 bp of sequence not contained in any of the other cDNA clones.

Figure 6 shows the composite cDNA sequence (SEQ ID NO:2) obtained from assembly of the partially overlapping cDNA clones shown in Figure 5. The predicted SCA2 protein product (SEQ ID NO:3) is shown below the DNA sequence. The stop codon for the SCA2 cDNA is indicated by \*. The locations of primers SCA2-A, SCA2-B, and SCA2-B14 are indicated by horizontal arrows. The splice site between primers SCA2-B and SCA2-B14 is indicated by a vertical arrow.

Figure 7 shows a partial amino acid sequence alignment comparison of ataxin-2 protein, the ataxin-2 related protein (A2RP), and the mouse SCA2 homologue in the region of strongest homology. Codon 1 corresponds to codon 155 in Figure 6 (SEQ ID NO:3).

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Figure 8 shows the genomic structure of the SCA2 gene.

#### DETAILED DESCRIPTION OF THE INVENTION

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The hereditary ataxias are a complex group of neurodegenerative disorders all characterized by varying abnormalities of balance attributed to dysfunction or pathology of the cerebellum and cerebellar pathways. In many of these disorders, dysfunction or structural abnormalities extend beyond the cerebellum, and may involve basal ganglia function, oculo-motor disorders and neuropathy. Among the inherited ataxias, the classification of dominant adult onset ataxias is particularly controversial with regard to nomenclature, associated findings and pathology. The dominant spinocerebellar ataxias (SCAs) represent a phenotypically heterogeneous group of disorders with a prevalence of familial cases of approximately 1 per 100,000. This group of disorders is also designated as olivo-ponto-cerebellar atrophies (OPCAs), although this term is too restrictive a pathological label.

The high phenotypic variability within single SCA pedigrees has made clinical classification of different forms of ataxia difficult. The gene causing SCA1 has been identified on CHR 6p and the SCA3 gene has been identified on CHR 14q. These diseases are caused by expansion of a CAG repeat in the coding region of the genes. However, many SCA pedigrees do not show linkage to CHR 6p or CHR 14q, confirming the presence of non-allelic heterogeneity. Subsequent genetic linkage studies have led to the identification of SCA loci on CHR12 and some families do not show linkage to either of the above three chromosomal regions.

Described in the instant specification is the construction of the BAC (Bacterial Artificial Chromosome) Shizuya et al., *Proc. Natl. Acad. Sci. USA* 89:8794-8797 (1992) contig and PAC (P1 Artificial Chromosome) of the SCA2 region and the isolation of a novel SCA2 gene from this contiguous map unit using a technique that screens for the presence of DNA trinucleotide repeats.

Sequence analysis of the DNA sequence flanking the CAG repeat revealed an open reading frame of 317 base pairs (Figure 2). A homology search of the amino acid sequence of this open reading frame (ORF) with genes registered in Genbank/EMBL and search of the TIGR database showed no homologous proteins or homologous genomic DNA sequences. Using reverse-transcribed PCR (polymerase chain reaction) with primers SCA1-A and SCA1-B, the genomic sequence containing the CAG repeat was shown to be expressed into mRNA. Subsequently, cDNA encoding human and mouse SCA2 has been isolated as described hereinafter in Examples 4 and 7, respectively.

Accordingly, the present invention provides isolated nucleic acids, which encode a novel mammalian SCA2 protein, and fragments thereof. Such nucleic acids can be obtained, for example, from human chromosome 12, specifically at the q24.1 locus, which is the site of mutation(s) that cause SCA2.

The term "nucleic acids" (also referred to as polynucleotides) encompasses RNA as well as single and double-stranded DNA and cDNA. As used herein, the phrase "isolated" means a nucleic acid that is in a form that does not occur in nature. One means of isolating a nucleic acid encoding an SCA2 polypeptide is to probe a mammalian genomic library with a natural or artificially designed DNA probe using methods well known in the art. DNA probes derived from the SCA2 gene are particularly

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useful for this purpose. DNA and cDNA molecules that encode SCA2 polypeptides can be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian (e.g., mouse, rat, rabbit, pig, and the like), or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Examples of nucleic acids are RNA, cDNA, or isolated genomic DNA encoding an SCA2 polypeptide. Such invention nucleic acids may include, but are not limited to, nucleic acids having substantially the same nucleotide sequence as nucleotides 163-4098 set forth in SEQ ID NO:2 (Figure 6), or at least nucleotides 163-657 or nucleotides 724-4098 of SEQ ID NO:2; or nucleotides 50-3454 of SEQ ID NO:4. In a preferred embodiment, invention nucleic acids include the same nucleotide sequence as nucleotides 163-4098 of SEQ ID NO:2, or include the same nucleotide sequence as nucleotides 50-3454 of SEQ ID NO:4.

As employed herein, the phrase "substantially the same nucleotide sequence" refers to DNA having sufficient homology to the reference polynucleotide, such that it will hybridize to the reference nucleotide under typical moderate stringency conditions. In one embodiment, nucleic acid molecules having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that of either SEQ ID NO:3, or SEQ ID NO:5. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% homology with respect to the reference nucleotide sequence. DNA having at least 70%, more preferably 80%, yet more preferably 90%, homology to the reference nucleotide sequence is preferred.

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This invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:4, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as "functionally equivalent nucleic acids". As used herein, the phrase "functionally equivalent nucleic acids" encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptides that are the same as those disclosed herein or that have conservative amino acid variations. For example, conservative variations include substitution of a non-polar residue with another non-polar residue, or substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as those that do not substantially alter the tertiary structure of the protein.

Further provided are nucleic acids encoding SCA2 polypeptides that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. Preferred nucleic acids encoding the invention polypeptide are comprised of nucleotides that encode substantially the same amino acid sequence set forth in SEQ ID NO:3 (Figure 6), or SEQ ID NO:5.

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As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences having at least about 70% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological properties characteristic of the protein defined by the reference amino acid sequence. Preferably, proteins having "substantially the same amino

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acid sequence" will have at least about 80%, more preferably 90% amino acid identity with respect to the reference amino acid sequence (SEQ ID NO:3 or SEQ ID NO:5); with greater than about 95% amino acid sequence identity being especially preferred.

Alternatively, preferred nucleic acids encoding the invention polypeptide(s) hybridize under moderately stringent, preferably high stringency, conditions to substantially the entire sequence, or substantial portions (i.e., typically at least 15-30 nucleotides) of the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2 (Figure 6) or SEQ ID NO:4.

Stringency of hybridization, as used herein, refers to conditions under which polynucleotide hybrids are stable. As known to those of skill in the art, the stability of hybrids is a function of sodium ion concentration and temperature (See, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual* 2d Ed. (Cold Spring Harbor Laboratory, (1989); incorporated herein by reference). Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art.

As used herein, the phrase "moderately stringent" hybridization refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60%, preferably about 75%, more preferably about 85%, homology (i.e., identity) to the target DNA; with greater than about 90% homology to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring

Harbor Laboratory Press, (1989)) are well known to those of skill in the art as are other suitable hybridization buffers.

5           Also provided are isolated SCA2 peptides, polypeptides(s) and/or protein(s), or fragments thereof, encoded by the invention nucleic acids.

10           As used herein, the term "isolated" means a protein molecule free of cellular components and/or contaminants normally associated with a native *in vivo* environment. Invention polypeptides and/or proteins include any isolated natural occurring allelic variant, as well as recombinant forms thereof. The SCA2  
15 polypeptides can be isolated using various methods well known to a person of skill in the art. The methods available for the isolation and purification of invention proteins include, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography.  
20 Other well-known methods are described in Deutscher et al., *Guide to Protein Purification: Methods in Enzymology* Vol. 182, (Academic Press, (1990)), which is incorporated herein by reference. Alternatively, the isolated polypeptides of the present invention can be  
25 obtained using well-known recombinant methods as described, for example, in Sambrook et al., *supra.*, (1989).

30           An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding the SCA2 in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again  
35 using well-known methods. Invention polypeptides can be isolated directly from cells that have been transformed

with expression vectors, described below in more detail. The invention polypeptide, biologically active fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic

5 polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

10 As used herein, the phrase "SCA2" refers to substantially pure native SCA2 protein, or recombinantly expressed/produced (i.e., isolated or substantially pure) proteins, including variants thereof encoded by mRNA generated by alternative splicing of a primary  
15 transcript, and further including fragments thereof which retain native biological activity. Preferred invention polypeptides are those that contain substantially the same amino acid sequence set forth in SEQ ID NO:3 (Figure 6), or at least amino acids 1-165 or amino acids 188-1312  
20 of SEQ ID NO:3, or include substantially the same amino acid sequence set forth in SEQ ID NO:5. As used herein, the phrase "functional polypeptide" means a SCA2 that can produce an anti-SCA2 antibody that binds to the native SCA2 protein or to the amino acid sequence set forth in  
25 SEQ ID NO:3 (Figure 6), or SEQ ID NO:5. In a preferred embodiment, invention polypeptides include the same amino acid sequence as set forth in SEQ ID NO:3 or SEQ ID NO:5.

Modification of the invention nucleic acids,  
30 polypeptides or proteins with the following phrases: "recombinantly expressed/produced", "isolated", or "substantially pure", encompasses nucleic acids, peptides, polypeptides or proteins that have been produced in such form by the hand of man, and are thus  
35 separated from their native *in vivo* cellular environment.

As a result of this human intervention, the recombinant nucleic acids, polypeptides and proteins of the invention

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are useful in ways that the corresponding naturally occurring molecules are not, such as identification of selective drugs or compounds.

5                Sequences having "substantially the same  
sequence" homology are intended to refer to nucleotide  
sequences that share at least about 75%, preferably about  
80%, yet more preferably about 90% identity with  
invention nucleic acids; and amino acid sequences that  
10 typically share at least about 75%, preferably about 85%,  
yet more preferably about 95% amino acid identity with  
invention polypeptides. It is recognized, however, that  
polypeptides or nucleic acids containing less than the  
above-described levels of homology arising as splice  
15 variants or that are modified by conservative amino acid  
substitutions, or by substitution of degenerate codons  
are also encompassed within the scope of the present  
invention.

20                The present invention provides the isolated  
polynucleotide encoding SCA2 operatively linked to a  
promoter of RNA transcription, as well as other  
regulatory sequences. As used herein, the phrase  
"operatively linked" refers to the functional  
25 relationship of the polynucleotide with regulatory and  
effector sequences of nucleotides, such as promoters,  
enhancers, transcriptional and translational stop sites,  
and other signal sequences. For example, operative  
linkage of a polynucleotide to a promoter refers to the  
30 physical and functional relationship between the  
polynucleotide and the promoter such that transcription  
of DNA is initiated from the promoter by an RNA  
polymerase that specifically recognizes and binds to the  
promoter, and wherein the promoter directs the  
35 transcription of RNA from the polynucleotide.

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15            Vectors that contain both a promoter and a  
cloning site into which a polynucleotide can be  
operatively linked are well known in the art. Such  
vectors are capable of transcribing RNA *in vitro* or *in*  
20 *vivo*, and are commercially available from sources such as  
Stratagene (La Jolla, CA) and Promega Biotech (Madison,  
WI). In order to optimize expression and/or *in vitro*  
transcription, it may be necessary to remove, add or  
alter 5' and/or 3' untranslated portions of the clones to  
eliminate extra, potential inappropriate alternative  
25 translation initiation codons or other sequences that may  
interfere with or reduce expression, either at the level  
of transcription or translation. Alternatively,  
consensus ribosome binding sites can be inserted  
immediately 5' of the start codon to enhance expression.  
30 (See, for example, Kozak, *J. Biol. Chem.* 266:19867  
(1991)). Similarly, alternative codons, encoding the  
same amino acid, can be substituted for coding sequences  
of the SCA2 polypeptide in order to enhance transcription  
(e.g., the codon preference of the host cell can be  
35 adopted, the presence of G-C rich domains can be reduced,  
and the like).

Also provided are vectors comprising invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA.

Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Other means are well known and available in the art.

Further provided are vectors comprising nucleic acids encoding SCA2 polypeptides, adapted for expression in a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), a mammalian cell and other animal cells. The vectors additionally comprise the regulatory elements

necessary for expression of the nucleic acid in the bacterial, yeast, amphibian, mammalian or animal cells so located relative to the nucleic acid encoding SCA2 polypeptide as to permit expression thereof.

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As used herein, "expression" refers to the process by which nucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the nucleic acid is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eucaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook et al. *supra*). Similarly, a eucaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the invention polypeptide.

The present invention provides transformed host cells that recombinantly express SCA2 polypeptides. An example of a transformed host cell is a mammalian cell comprising a plasmid adapted for expression in a mammalian cell. The plasmid contains nucleic acid encoding an SCA2 polypeptide and the regulatory elements necessary for expression of invention proteins. Various mammalian cells may be utilized as hosts, including, for example, mouse fibroblast cell NIH3T3, CHO cells, HeLa

cells, Ltk- cells, etc. Expression plasmids such as those described *supra* can be used to transfect mammalian cells by methods well known in the art such as, for example, calcium phosphate precipitation, DEAE-dextran, electroporation, microinjection or lipofection.

The present invention provides nucleic acid probes comprising nucleotide sequences capable of specifically hybridizing with sequences included within nucleic acids encoding SCA2 polypeptides, for example, a coding sequence included within the nucleotide sequence shown in SEQ ID NO:2 (Figure 6), or SEQ ID NO:4. In a preferred embodiment, the probe is derived from the nucleic acid sequence set forth in SEQ ID NO:2, or at least nucleotides 163-657 or nucleotides 724-4098 of SEQ ID NO:2; or SEQ ID NO:4. Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences within the ORF, and the like. Full-length or fragments of cDNA clones encoding SCA2 can also be used as probes for the detection and isolation of related genes. As used herein, an invention "probe" or invention oligonucleotide is a single-stranded DNA or RNA that has a sequence of nucleotides that includes at least about 15 contiguous bases up to the full length coding region of SEQ ID NO:2 or SEQ ID NO:4. Preferably an invention probe is at least about 30 contiguous bases, more preferably at least about 50, yet more preferably at least about 100, with about 300 contiguous bases up to the full length coding region of SEQ ID NO:2 and SEQ ID NO:4 being especially preferred. When fragments are used as probes, preferably the cDNA sequences will be from the carboxyl end-encoding portion of the cDNA, and most preferably will include predicted transmembrane domain-encoding portions of the cDNA sequence. Transmembrane domain regions can be predicted based on hydrophathy analysis of the deduced amino acid sequence using, for

example, the method of Kyte and Doolittle, *J. Mol. Biol.* 157:105 (1982).

As used herein, the phrase "specifically hybridizing" encompasses the ability of a polynucleotide to recognize a sequence of nucleic acids that are complementary thereto and to form double-helical segments via hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable agent, such as a radioisotope, a fluorescent dye, and the like, to facilitate detection of the probe. Invention probes are useful to detect the presence of nucleic acids encoding the SCA2 polypeptide. For example, the probes can be used for *in situ* hybridizations in order to locate biological tissues in which the invention gene is expressed. Additionally, synthesized oligonucleotides complementary to the nucleic acids of a nucleotide sequence encoding SCA2 polypeptide are useful as probes for detecting the invention genes, their associated mRNA, or for the isolation of related genes using homology screening of genomic or cDNA libraries, or by using amplification techniques well known to one of skill in the art.

Also provided are antisense oligonucleotides having a sequence capable of binding specifically with any portion of an mRNA that encodes SCA2 polypeptides so as to prevent or inhibit translation of the mRNA. The antisense oligonucleotide may have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding SCA2 polypeptides. As used herein, the phrase "binding specifically" encompasses the ability of a nucleic acid sequence to recognize a complementary nucleic acid sequence and to form double-helical segments therewith via the formation of hydrogen bonds between the

complementary base pairs. An example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogs of nucleotides.

5 Compositions comprising an amount of the antisense oligonucleotide, described above, effective to reduce expression of SCA2 polypeptides by passing through a cell membrane and binding specifically with mRNA encoding SCA2 polypeptides so as to prevent translation  
10 and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. The acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is  
15 thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind to a cell-type specific receptor.

Antisense oligonucleotide compositions are  
20 useful to inhibit translation of mRNA encoding invention polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding SCA2 polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit  
25 expression of SCA2 associated genes in a tissue sample or in a subject.

In accordance with another embodiment of the invention, kits for detecting mutations and aneuploidies  
30 in chromosome 12 at locus q24.1 comprising at least one invention probe or antisense nucleotide.

The present invention provides means to modulate levels of expression of SCA2 polypeptides by  
35 employing synthetic antisense oligonucleotide compositions (hereinafter SAOC) which inhibit translation of mRNA encoding these polypeptides. Synthetic

08981998-051198

oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the SCA2 coding strand or nucleotide sequences shown in SEQ ID NO:2, or SEQ ID NO:4. The SAOC is designed to be stable in the blood stream for administration to a subject by injection, or in laboratory cell culture conditions. The SAOC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOC which render it capable of passing through cell membranes, for example, by designing small, hydrophobic SAOC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SAOC into the cell. In addition, the SAOC can be designed for administration only to certain selected cell populations by targeting the SAOC to be recognized by specific cellular uptake mechanisms which bind and take up the SAOC only within select cell populations.

For example, the SAOC may be designed to bind to a receptor found only in a certain cell type, as discussed *supra*. The SAOC is also designed to recognize and selectively bind to target mRNA sequence, which may correspond to a sequence contained within the sequence shown in SEQ ID NO:2, or SEQ ID NO:4. The SAOC is designed to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of translation-regulating factors or ribosomes, or inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SAOCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., TIPS, 10:435

(1989) and Weintraub, *Sci. American*, January (1990), pp.40; both incorporated herein by reference).

The present invention also provides  
5 compositions containing an acceptable carrier and any of  
an isolated, purified SCA2 polypeptide, an active  
fragment thereof, or a purified, mature protein and  
active fragments thereof, alone or in combination with  
each other. These polypeptides or proteins can be  
10 recombinantly derived, chemically synthesized or purified  
from native sources. As used herein, the term  
"acceptable carrier" encompasses any of the standard  
pharmaceutical carriers, such as phosphate buffered  
saline solution, water and emulsions such as an oil/water  
15 or water/oil emulsion, and various types of wetting  
agents.

Further provided are anti-SCA2 antibodies  
having specific reactivity with SCA2 polypeptides of the  
20 present invention. Active fragments of antibodies are  
encompassed within the definition of "antibody".  
Invention antibodies can be produced by methods known in  
the art using invention polypeptides, proteins or  
portions thereof as antigens. For example, polyclonal  
25 and monoclonal antibodies can be produced by methods well  
known in the art, as described, for example, in Harlow  
and Lane, *Antibodies: A Laboratory Manual* (Cold Spring  
Harbor Laboratory (1988)), which is incorporated herein  
by reference. Invention polypeptides can be used as  
30 immunogens in generating such antibodies. Alternatively,  
synthetic peptides can be prepared (using commercially  
available synthesizers) and used as immunogens. Amino  
acid sequences can be analyzed by methods well known in  
the art to determine whether they encode hydrophobic or  
35 hydrophilic domains of the corresponding polypeptide.  
Altered antibodies such as chimeric, humanized, CDR-  
grafted or bifunctional antibodies can also be produced

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by methods well known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook et al., *supra.*, and Harlow and Lane, *supra.* Both anti-peptide and anti-fusion protein antibodies can be used. (see, for example, Bahouth et al., *Trends Pharmacol. Sci.* 12:338 (1991); Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley and Sons, NY (1989) which are incorporated herein by reference).

10

Invention antibodies also can be used to isolate invention polypeptides. Additionally the antibodies are useful for detecting the presence of invention polypeptides, as well as analysis of chromosome localization, and structural as well as functional domains. Methods for detecting the presence of SCA2 polypeptides on the surface of a cell comprise contacting the cell with an antibody that specifically binds to SCA2 polypeptides, under conditions permitting binding of the antibody to the polypeptides, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of invention polypeptides on the surface of the cell. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo* imaging methods.

Immunological procedures useful for *in vitro* detection of target SCA2 polypeptides in a sample include immunoassays that employ a detectable antibody. Such immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures which are well known in the art. An antibody can be made detectable by various means well known in the art. For example, a detectable marker can be directly or indirectly attached

to the antibody. Useful markers include, for example, radionucleotides, enzymes, fluorogens, chromogens and chemiluminescent labels.

5 Further, invention antibodies can be used to modulate the activity of the SCA2 polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity  
10 for SCA2 polypeptides effective to block binding of naturally occurring ligands to invention polypeptides. A monoclonal antibody directed to an epitope of SCA2 polypeptide molecules present on the surface of a cell and having an amino acid sequence substantially the same  
15 as an amino acid sequence for a cell surface epitope of an SCA2 polypeptide shown in SEQ ID NO:3, or SEQ ID NO:5, can be useful for this purpose.

20 The present invention further provides transgenic non-human mammals that are capable of expressing nucleic acids encoding SCA2 polypeptides. Also provided are transgenic non-human mammals capable of expressing nucleic acids encoding SCA2 polypeptides so mutated as to be incapable of normal activity, i.e., do  
25 not express native SCA2. The present invention also provides transgenic non-human mammals having a genome comprising antisense nucleic acids complementary to nucleic acids encoding SCA2 polypeptides so placed as to be transcribed into antisense mRNA complementary to mRNA  
30 encoding SCA2 polypeptides, which hybridizes thereto and, thereby, reduces the translation thereof. The nucleic acid may additionally comprise an inducible promoter and/or tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell  
35 types. Examples of nucleic acids are DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID NO:2, or SEQ ID NO:4. An

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example of a non-human transgenic mammal is a transgenic mouse. Examples of tissue specificity-determining elements are the metallothionein promoter and the L7 promoter.

5

Animal model systems which elucidate the physiological and behavioral roles of SCA2 polypeptides are produced by creating transgenic animals in which the expression of the SCA2 polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an SCA2 polypeptide by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal. (See, for example, Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory, (1986)).

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Another technique, homologous recombination of mutant or normal versions of these genes with the native gene locus in transgenic animals, may be used to alter the regulation of expression or the structure of SCA2 polypeptides (see, Capecchi et al., *Science* 244:1288 (1989); Zimmer et al., *Nature* 338:150 (1989); which are incorporated herein by reference). Homologous recombination techniques are well known in the art. Homologous recombination replaces the native (endogenous) gene with a recombinant or mutated gene to produce an animal that cannot express native (endogenous) protein but can express, for example, a mutated protein which results in altered expression of SCA2 polypeptides.

In contrast to homologous recombination, microinjection adds genes to the host genome, without removing host genes. Microinjection can produce a

00981998-051198

transgenic animal that is capable of expressing both endogenous and exogenous SCA2 protein. Inducible promoters can be linked to the coding region of nucleic acids to provide a means to regulate expression of the transgene. Tissue specific regulatory elements can be linked to the coding region to permit tissue-specific expression of the transgene. Transgenic animal model systems are useful for *in vivo* screening of compounds for identification of specific ligands, i.e., agonists and antagonists, which activate or inhibit protein responses.

Invention nucleic acids, oligonucleotides (including antisense), vectors containing same, transformed host cells, polypeptides and combinations thereof, as well as antibodies of the present invention, can be used to screen compounds *in vitro* to determine whether a compound functions as a potential agonist or antagonist to invention polypeptides. These *in vitro* screening assays provide information regarding the function and activity of invention polypeptides, which can lead to the identification and design of compounds that are capable of specific interaction with one or more types of polypeptides, peptides or proteins.

In accordance with still another embodiment of the present invention, there is provided a method for identifying compounds which bind to SCA2 polypeptides. The invention proteins may be employed in a competitive binding assay. Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to SCA2 proteins. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention proteins.

In another embodiment of the invention, there is provided a bioassay for identifying compounds which modulate the activity of invention polypeptides.

According to this method, invention polypeptides are  
5 contacted with an "unknown" or test substance (in the presence of a reporter gene construct when antagonist activity is tested), the activity of the polypeptide is monitored subsequent to the contact with the "unknown" or test substance, and those substances which cause the  
10 reporter gene construct to be expressed are identified as functional ligands for SCA2 polypeptides.

In accordance with another embodiment of the present invention, transformed host cells that  
15 recombinantly express invention polypeptides can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the SCA2-mediated response (via reporter gene expression) in the presence and absence of test compound, or by  
20 comparing the response of test cells or control cells (i.e., cells that do not express SCA2 polypeptides), to the presence of the compound.

As used herein, a compound or a signal that  
25 "modulates the activity" of invention polypeptides refers to a compound or a signal that alters the activity of SCA2 polypeptides so that the activity of the invention polypeptide is different in the presence of the compound or signal than in the absence of the compound or signal.  
30 In particular, such compounds or signals include agonists and antagonists. An agonist encompasses a compound or a signal that activates SCA2 protein expression. Alternatively, an antagonist includes a compound or signal that interferes with SCA2 protein expression.  
35 Typically, the effect of an antagonist is observed as a blocking of agonist-induced protein activation. Antagonists include competitive and non-competitive

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antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for agonist binding. A non-competitive antagonist or blocker inactivates the function of the polypeptide by  
5 interacting with a site other than the agonist interaction site.

As understood by those of skill in the art, assay methods for identifying compounds that modulate  
10 SCA2 activity generally require comparison to a control. One type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the compound, with the distinction that the "control" cell or culture is not exposed to the  
15 compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence or absence of compound, by merely changing the external solution bathing the cell. Another type of "control" cell or culture may be a cell or  
20 culture that is identical to the transfected cells, with the exception that the "control" cell or culture do not express native proteins. Accordingly, the response of the transfected cell to compound is compared to the response (or lack thereof) of the "control" cell or  
25 culture to the same compound under the same reaction conditions.

In yet another embodiment of the present invention, the activation of SCA2 polypeptides can be  
30 modulated by contacting the polypeptides with an effective amount of at least one compound identified by the above-described bioassays.

In accordance with another embodiment of the  
35 present invention, there are provided methods for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

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detecting, in said subject, a genomic or transcribed mRNA sequence having an expanded CAG repeat at a location corresponding to between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6).

The number of CAG repeats required to indicate spinocerebellar Ataxia Type 2 is substantially above normal, preferably at least about 10-15 CAG repeats above normal, with at least 13 CAG repeats above normal being especially preferred. A normal amount of CAG repeats in the SCA2 gene (SEQ ID NO:2) has been found to be about 22, while 23 CAG repeats is occasionally observed. Thus, in a preferred diagnostic method, at least about 35 CAG repeats are detected between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), with the detection of 37 CAG repeats being especially preferred.

Although expansion of trinucleotide repeats is now recognized as an important mutational mechanism in humans and SCA2 represents the 6th disease in which expansion of a CAG trinucleotide repeat causes disease, there are several features of the SCA2 repeat that appear to be unique. In the other five CAG expansion diseases, the CAG repeats on normal chromosomes are highly polymorphic. Multiple alleles are detected and repeat sizes on normal chromosomes range from a low of 7 repeats in DRPLA to 40 repeats in SCA3/MJD. Heterozygosity for these CAG repeats in the normal population are in the range of 0.80 and above. It has been suggested that the extended normal alleles represent founder alleles which are predisposed to expansion:

The SCA2 repeat is highly unusual, because only two alleles are observed in the normal population. A common allele with 22 repeats is found on 92% of chromosomes, a rare second allele in 8% of chromosomes.

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functionally important domains adjacent to polyglutamine tracts may provide the potential for novel strategies to analyze the function of ataxin-2. A gain of function for the mutated ataxin-2 is supported by the fact that  
5 transcripts coding for mutated alleles are detected by RT-PCR.

Expansion of the SCA2 repeat appears to be a common cause of a dominant SCA phenotype in non-  
10 Portuguese patients. When samples from 45 families with SCA were screened, samples from 8 independent pedigrees showed expansion of the SCA2 repeat. It has been suggested that there are features specific to SCA2, but this assessment was limited to families large enough to  
15 be studied by linkage analysis. A better assessment of the range of SCA2 phenotypes is now possible due to the ability to test small families and single cases. In our patient sample, most patients had a 'typical' SCA phenotype, but some patients had been classified as  
20 having an MJD phenotype and others showed a prominent dementia.

When performing direct testing for SCA2 mutations, great caution has to be exercised when  
25 interpreting the presence of expanded SCA2 alleles on polyacrylamide gels. A variable number of unrelated PCR fragments may be seen that are in the size range of expanded SCA2 repeats. Although these bands lack the typical 'shadow' bands seen when di- or trinucleotide  
30 repeats are amplified, they may interfere with the interpretation in some samples. It is therefore recommended to confirm the presence of an expanded allele by Southern blotting and hybridization with a (CAG)<sub>10</sub> oligonucleotide.

In yet another embodiment of the present invention, there are provided methods for diagnosing spinocerebellar Ataxia Type 2, said method comprising:

5 a) contacting nucleic acid obtained from a subject suspected of having SCA2 with primers that amplify at least a nucleic acid fragment of SEQ ID NO:2 containing nucleotides 658-723 of SEQ ID NO:2, under conditions suitable to form a detectable amplification product; and

10 b) detecting an amplification product containing substantially expanded CAG repeats above normal, whereby said detection indicates that said subject has SCA2.

15 As indicated above, substantially expanded CAG repeats have at least about 10-15 CAG repeats above normal, with at least 13 CAG repeats above normal being especially preferred. Thus, in a preferred diagnostic method, at least about 35 CAG repeats are detected  
20 between nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), with the detection of 37 CAG repeats being especially preferred.

In accordance with another embodiment of the  
25 present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid in a suitable packaging material. In one embodiment, the diagnostic nucleic acids are derived from SEQ ID NO:2 (Figure 6), preferably derived from  
30 nucleotides 163-657 and nucleotides 724-4098, with primers SCA2-A and SCA2-B being especially preferred. In another embodiment, the diagnostic nucleic acids are derived from SEQ ID NO:4. Invention diagnostic systems are useful for assaying for the presence or absence of  
35 the extended CAG repeat sequence between nucleotides 657 and 724 of SEQ ID NO:2 in the SCA2 gene in either genomic

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DNA or in transcribed nucleic acid (such as mRNA or cDNA) encoding SCA2.

5 A suitable diagnostic system includes at least one invention nucleic acid, preferably two or more invention nucleic acids, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art  
10 can readily incorporate invention nucleic probes and/or primers into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein.

15 As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as invention nucleic acid probes or primers, and the like. The packaging material is constructed by well known methods,  
20 preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention nucleic acids can be used for detecting a particular extended CAG repeat sequence between the region of genomic DNA corresponding to  
25 nucleotides 657 and 724 of SEQ ID NO:2 (Figure 6), thereby diagnosing the presence of, or a predisposition for, spinocerebellar ataxia type 2. In addition, the packaging material contains instructions indicating how the materials within the kit are employed both to detect  
30 a particular sequence and diagnose the presence of, or a predisposition for, spinocerebellar ataxia type 2.

The packaging materials employed herein in relation to diagnostic systems are those customarily  
35 utilized in nucleic acid-based diagnostic systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the

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like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a

5 contemplated nucleic acid, oligonucleotide or primer, or it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

10 "Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, 15 temperature, buffer conditions, and the like.

20 All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting examples.

25 The invention will now be described in greater detail with reference to the following non-limiting examples.

#### Materials and Methods

30 Unless otherwise stated, the present invention was performed using standard procedures, as described, for example in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1982); Sambrook et al., Molecular Cloning: A Laboratory Manual (2 ed.), Cold 35 Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989); Davis et al., Basic Methods in

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*Molecular Biology*, Elsevier Science Publishing, Inc., New York, USA (1986); or *Methods in Enzymology: Guide to Molecular Cloning Techniques* Vol.152, S. L. Berger and A. R. Kimmerl Eds., Academic Press Inc., San Diego, USA (1987)).

Libraries. Yeast artificial chromosome (YAC) clones were obtained from the CEPH mega-YAC library and grown under standard conditions (Cohen et al., *Nature* 366:689-701 (1993)). P1 artificial chromosome (PAC) library construction. A 3X human PAC library, designated RPCI-1 (Ioannou et al., *Hum. Genet.* 219-220 (1994b)) was constructed as described (Ioannou et al., *Nat. Genet.* 6:84-89 (1994a)). The library was arrayed in 384 well dishes. Pools from portion of the library were screened by PCR with AFM154TC5 (D12S1333) and AFMa128yf1 (D12S1332). Subsequently, STSS generated by sequencing of clones using vector primers were used as hybridization probes to gridded colony filters of the PAC library.

YAC DNA preparation. YAC clones were grown in selective media, pelleted and resuspended in 3 ml 0.9 M sorbitol, 0.1M EDTA pH 7.5, then incubated with 100 U of lytocase (Sigma) at 37°C for 1 hour. After centrifugation for 5 minutes at 5,000 rpm pellets were resuspended in 3 ml 50 mM Tris pH 7.45, 20 mM EDTA three-tenth ml 10% SDS was added and the mixture was incubated at 65°C for 30 minutes. One ml of 5 M potassium acetate was added and tubes were left on ice for 1 hour, then centrifuged at 10,000 rpm for 10 minutes. Supernatant was precipitated in 2 volumes of ethanol and pelleted at 6,000 rpm for 15 minutes. Pellets were resuspended in TE, treated with RNase and reextracted with phenol-chloroform.

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*Analysis by pulsed-field gel electrophoresis.*

Agarose plugs of yeast cells containing total YAC DNA were prepared (Larin and Lehrach, *Genet. Res.* 56:203-208 (1990)) and subjected to pulsed-field gel separation on 5 1% SeaKem agarose gels in 0.5X TBE using the CHEF DRII Mapper (Bio-Rad). PAC and BAC clones were sized after digestion with XbaI and NotI. Gels were blotted onto Magna NT Nylon membranes using alkaline blotting, UV 10 cross linked and baked at 80°C for two hours. Membranes were hybridized with total human DNA, washed according to standard procedures, and exposed to Kodak XAR5 film. The sizes of individual clones were determined by comparison to their relative positions with molecular weight standards.

15

*Analysis by fluorescence in situ hybridization*

(FISH). PAC or BAC clones were biotinylated by nicktranslation in the presence of biotin-14-dATP using the BioNick Labeling Kit (Gibco-BRL). FISH was performed 20 essentially as described (Korenberg et al., *Cytogenet Cell Genet.* 69:196-200 (1995)). Briefly, 400 ng of probe DNA was mixed with 8 ng of human Cot 1 DNA (Gibco-BRL) and 2 ug of sonicated salmon sperm DNA in order to suppress possible background produced from repetitive 25 human sequences as well as yeast sequences in the probe. The probes were denatured at 75°C, preannealed at 37°C for one hour, and applied to denatured chromosome slides prepared from normal male lymphocytes (Korenberg et al., 1995, *supra*). Post-hybridization washes were performed 30 at 40°C in 2X SSC/50% formamide followed by washes in 1X SSC at 50°C. Hybridized DNAs were detected with avidin-conjugated fluorescent isothiocyanate (Vector Laboratories). One amplification was performed by using biotinylated anti-avidin. For distinguishing chromosome 35 subbands precisely, a reverse banding technique was used, which was achieved by chromomycin A3 and distamycin A

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double staining (Korenberg et al., 1995, *supra*). The color images were captured by using a Photometrics Cooled-CCD camera and BDS image analysis software (Oncor Imaging, Inc.).

5

*PAC and BAC DNA preparation.* Selected clones were grown overnight in LB media containing 12.5 µg/ml kanamycin for PACs and 12.5 µg/ml chloramphenicol for BACs. DNAs were prepared by the alkaline lysis method. 10 PAC DNAs were digested with *NotI* and subjected to pulsed-field gel electrophoresis. Sizes were determined relative to λ concatamers.

*Southern blot analysis.* Gel electrophoresis of 15 DNA was carried out on 0.8% agarose gels in 1x TBE. Transfer of nucleic acids to Nybond N+ nylon membrane (Amersham) was performed according to the manufacturer's instruction. Probes were labelled using RadPrime Labeling System (BRL). Hybridization was carried out at 20 42°C for 16 hours in 50% formamide, 5x SSPE, 5x Denhardt's 0.1% SDS, 100 mg/ml denatured salmon sperm DNA. The filters were washed once in 1x SSC, 0.1% SDS at room temperature for 20 minutes, and twice in 0.1x SSC, 0.1% SDS for 20 minutes at 65°C. The blots were exposed 25 onto X-ray film (Kodak, X-OMAT-AR).

*Sequencing of PAC endclones.* PAC clones were inoculated into 500 ml of LB/kanamycin and grown overnight. DNAs were isolated using QIAGEN columns 30 according to the vendors protocol with one additional phenol/chloroform/isoamylalcohol extraction followed by one additional chloroform/isoamylalcohol extraction. Clones were sequenced using the Gibco-BRL cycle sequencing kit with standard T7 and SP6 primers.

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### Hybridization of (CAG)<sub>10</sub> oligonucleotides.

Eighty ng of oligonucleotide were 5' end-labeled and hybridized overnight at 42°C in buffer containing 1 M NaCl, 0.05 M Tris HCl pH7, 5.5 mM EDTA, 0.1 % SDS, 1X Denhardt's solution and 200 µg/ml denatured salmon sperm DNA. Filters were washed 2 times with 2X SSC, 0.1% SDS at 55°C and exposed to Kodak X-ray film for 24 hours, and subsequently washed at 65°C, followed by additional exposure to X-ray film.

10

*Regression Analysis.* The data were fit using the Statistical Analysis Software (SAS) package version 3.10 using the Secant Method (Ralston et al, 1978, Technometrics, 20:7-14). The regression equation was  $y = A \cdot \exp(-ax)$ , where y gives the age of onset and x the number of CAG repeats. The conversion criteria were met with the mean square error of 76.598. The value of parameters are as follows:  $A = 1171.583$ ,  $a = 0.091$ .

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### EXAMPLE 1

#### Physical Map of the SCA2 region

BAC library construction of total human genomic DNA was performed as described in Shizuya et al., *Proc. Natl. Acad. Sci. USA* 89:8794-8797 (1992). BAC clones were screened by PCR using STSs (D12S1228, S29, S32, S33). Insert size of clones was measured by running pulsed-field gel electrophoresis after digesting DNA with NotI.

25

30

The marker AFMa128yf1 (D12S1332) which was non-recombinant in several SCA2 pedigrees served as the starting point to assemble a PAC contig. This was done by screening PCR pools of a 3x human PAC library (Ioannou et al., 1994). Two clones were positive for this STS (Fig. 1). Single copy sequences from PAC ends were obtained from P168L1 and used to extend this contig.

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Subsequent 'walking steps, however, were undertaken by hybridizing PCR-generated STS fragments to gridded membranes of the 3x PAC library and the 1x total human genome BAC library (Research Genetics).

5

In a similar fashion, a second contig was established starting with the telomeric flanking marker AFM154tc5 (D12S1333). A total of two clones were identified by screening of PCR pools. After several walking steps, overlap of the two contigs was established by shared STSs (Fig. 1) and by shared restriction fragments (data not shown). All STSs shown in Fig. 1 were mapped back to human chromosome 12 by PCR analysis of a human/Chinese hamster somatic hybrid cell line, HHW582, which contains CHR 12 as the only human chromosome, and by analysis of a chromosome 12 specific lambda library, LL12NS01 (both from Coriell Cell Repositories). Map position in 21q24.1 for clones B295C05, P191C5 and P65I22 was confirmed using FISH (Fig. 1b).

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At the same time contigs were constructed for the other flanking markers AFM240wel (D12S1328), AFM291xe9 (D12S1329), and markers WI-4176 and WI-6850 (data not shown). These contigs did not overlap with one another, nor with the AFM128yf1/AFM154tc5 contig.

25

All PAC and BAC clones were sized by pulsed-field electrophoresis after digestion with NotI. Overlap of clones was initially determined by shared STS content, and subsequently confirmed by hybridization of selected clones to Southern blots of NotI/XbaI digests of clones.

30

The dense localization of STSs allowed the precise positioning of YACs that had been identified by screening of PCR pools of the CEPH mega-YAC library with either AFM128yf1 or AFM154tc5. The only YAC that was

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positive for both AFMa128yf1 (D12S1332) and AFM154tc5, Y884\_h\_11, contained an approximately 200 kb interstitial deletion. A small portion of this deletion was not covered by any of the other YAC clones.

5

## EXAMPLE 2

Identification of SCA2-related trinucleotide repeats

Since we had observed marked anticipation in one pedigree with SCA2, we identified clones containing trinucleotide repeats. EcoRI digests of a minimal tiling path of PAC clones were hybridized with a (CAG)<sub>10</sub> nucleotide, as well as other trinucleotide permutations. Three CAG positive bands of distinct sizes were identified in the contig.

PAC clone P65I22 was digested with Sau3A and subcloned into the pBluescript SK (+) phagemid (Stratagene). After transfection into DH5 $\alpha$ , bacterial colonies were screened for poly-CAG containing inserts using the methods described above. Positive clones were sequenced using the Circum Vent cycle sequencing kit (New England Biolabs) with end-labeled T3 and T7 primers. However, no reliable sequence could be obtained from the initial plasmid PL65I22. Therefore, this plasmid was digested with BssHII, recloned into the pBluescript plasmid, and CAG-positive clones sequenced with primers corresponding to the following nucleotides of the vector sequence (primer A: 828-848, primer B: 547-565). The sequence of this plasmid, designated PL65I22B, allowed the generation of primers SCA2-A and SCA2-B, which were used to confirm the sequence flanking the CAG repeat.

Plasmid PL65I22B containing an extended CAG repeat that appeared to be embedded into a long open reading frame (ORF) (Figure 2; SEQ ID NO:1). Sequence analysis of this plasmid appeared to be extremely

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difficult due to the abundant presence of premature terminations (see below). The CAG repeat in PL65I22B was twice interrupted and had the following structure (CAG)<sub>8</sub>CAA(CAG)<sub>4</sub>CAA(CAG)<sub>8</sub>. Four additional PAC clones and one BAC clone contained the SCA2 repeat, and all clones had 22 repeats with two CAA interruptions. Analysis of the genomic DNA sequence flanking the CAG repeat suggested the presence of an open reading frame (see also Figure 6) and a potential splice site 3' of the CAG repeat (vertical arrow in Figure 2).

The difficulties encountered in sequencing this region suggested that stable secondary structures might be formed in this GC-rich region. Previous analysis of trinucleotide repeats predisposed to expansion had suggested that these regions are predicted to form hairpin structures. We used an up-dated version of the DNA-FOLD Program (SantaLucia et al., 1996, Biochemistry, 35:3555-3562) for secondary structure predictions.

Subsequent analysis of the sequence flanking the CAG repeat using the OLIGO Program indicated that it contained several palindromic sequences predicted to form hairpin loops. Despite the predicted hairpin structures sufficient sequence information was generated to design primers flanking the CAG repeat for the PCR analysis of patient samples.

### Example 3

#### Genomic analysis of an extended CAG SCA2 repeat

Using primer pairs SCA2-A and B, genomic DNAs from normal controls and SCA2 patients were amplified and separated by agarose gel electrophoresis. The best results were obtained at an annealing temperature of 63°C with denaturation times of 90 sec.

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Eighty ng each of primers SCA2-A (5'-GGG CCC CTC ACC ATG TCG-3') and SCA2-B (5'-CGG GCT TGC GGA CAT TGG-3') were added to 20 ng of human DNA with standard PCR buffer and nucleotide concentrations. After an

5 initial denaturation at 95°C for 5 minutes, 35 cycles were repeated with denaturation at 96°C for 1.5 minutes, an annealing temperature of 63°C for 30 seconds, extension at 72°C for 1.5 minutes, and a final extension of 5 minutes at 72°C.

10

PCR products obtained by PCR amplification of genomic DNAs were separated by electrophoresis through 2% agarose gels in 1x TBE buffer at 10 V/cm. Gels were transferred to nylon membranes (MSI, Westborough, MA) using standard procedures for Southern blotting. Membranes were hybridized with a (CAG)<sub>10</sub> oligonucleotide and processed as described above.

On agarose electrophoresis, a single band of approximately 130 bp was detected in 20 normal individuals, although occasionally two closely spaced bands could be observed. In contrast, all 15 patients with SCA2 from 3 independent families showed one allele in the normal size range and a larger allele ranging from approximately 190 to 250 bp. Southern blot analysis confirmed that both alleles contained CAG repeats.

To determine the exact sizes of amplified fragments, DNAs from SCA2 patients and 50 normal individuals were amplified and PCR products separated by polyacrylamide gel electrophoresis. A common allele of 22 repeats and a less frequent allele of 23 repeats were observed on normal chromosomes (Figure 3). The allele frequencies were 0.92 for the smaller and 0.08 for the larger allele. In patients from three independent SCA2 pedigrees, however, extended alleles ranging from 36 to 52 repeats were observed (Figure 3). Once expanded to

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the pathologic range, the SCA2 repeat was moderately unstable and further expansion by 2 to 9 repeat units was observed during meiosis (Figure 3). There was great variability of the age of onset for a given repeat length, especially for disease alleles with 36-40 repeats (Figure 4). Due to the heterogeneous variance of age of onset we used non-linear regression, and an exponential function was successfully fitted (see methods and Figure 4). The smallest expansion of 36 repeats was seen in two men with disease onset at ages 37 and 44. The longest expansion of 52 repeats was seen in a boy with disease onset at 9 years of age.

Sequence analysis of ten normal alleles revealed that the common normal allele with 22 repeats contained the two CAA interruptions that were also detected in plasmid PL65I22B. The less frequent normal allele with 23 repeats had lost the 5' CAA interruption, and contained an additional CAG repeat at the 5'-end of the repeat. In three expanded alleles that were isolated from SCA2 patients the CAG repeat lacked any interruptions.

To determine the frequency of mutation in the SCA2 gene in non-Portuguese patients we screened DNAs from 45 independent families with autosomal dominant SCAs. Expansion of the SCA2 repeat was detected in six families. In this set of families, SCA2 expansion was twice as common as expansion in the SCA1 gene. In addition to individuals with a 'typical' SCA phenotype, expansion of the SCA2 repeat was detected in a pedigree with a MJD phenotype and one family with SCA and marked dementia.

## EXAMPLE 4

Isolation of human SCA2 cDNA

cDNA library screen: <sup>32</sup>P-labeled probes were generated by

5 PCR amplification of plasmid P65I22B using the following  
 primer pair: 65A3: 5'CCGCGGCTGCCAATGTCC, 65B5:  
 5'GTAACCGTTCGGCGCCCG. A second probe was generated using  
 primers 65A6: 5'GGCTCCCGGCGGCTCCTT, 65B6:  
 5'TGCTGCTGCTGCTGGGGCTTCAG. Screening of the trisomy 21  
 10 fetal brain cDNA library and the Stratagene adult human  
 frontal cortex cDNA Lambda Zap II library was performed  
 using the amplification products generated from plasmid  
 P65I22B. Phages were plated to an average density of  $1 \times 10^5$   
 per 150 cm<sup>2</sup> plate. Plaque lifts of 20 plates ( $2 \times 10^6$   
 15 phages) were made using duplicated nylon membranes  
 (Duralose-UV, Stratagene). Hybridization and excision  
 were performed according to the manufacturer's protocol.  
 Hybridized membranes were washed to a final stringency of  
 0.2x SSC, 0.1x SDS at 65C. The filters were exposed  
 20 overnight onto X-ray film. Excised phagemids were grown  
 overnight in 5ml LB medium containing 50 ug/ml of  
 ampicillin.

Using PCR-generated fragments containing

25 nucleotides 39-237 and 262 to 397 (according to the  
 sequence shown in Figure 2) we initially screened a human  
 adult frontal cortex library (Stratagene). Through  
 screening of  $0.8 \times 10^6$  clones, two positive clones, S1 and  
 S2, were identified. To obtain additional clones,  $2 \times 10^6$   
 30 clones of a human fetal brain library generated from a  
 fetus with trisomy 21 (Yamakawa et al., 1995, Hum. Mol.  
Genet., 4:709-716) were screened using the same PCR-  
 generated fragments. A total of 15 clones were obtained,  
 all of which were partially sequenced to determine  
 35 alignment of clones. These clones appeared to belong to  
 a total of two classes of clones (designated F1.1 through  
 F1.7 and F2.1 through F2.8) that contained long portions

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of the 3' untranslated region and a poly-A tail (Figure 5). Both classes of clones extended 40 and 265 bp 5' of the CAG repeat in the coding region of the SCA2 gene.

5 To obtain cDNA sequence for the 5' end of the SCA2 coding region, placental poly-T selected placental mRNAs (Clontech) were transcribed with MMLV reverse transcriptase and amplified with the following primer pairs: SCA2-A30: 5'-CCGCCCCGCTCCTCACGTGT, SCA2-A31: 5'-ACCCCCGAGAAAGCAACC, SCA2-B30: 5'-CCGTTGCCGTTGCTACCA. The sequences for primers SCA2-A30 and A31 were obtained from genomic sequence, and are located 5' to the stop codon preceding the putative initiator methionine. The sequence for SCA2-B30 was obtained from the 5' end of cDNA clones F1.1 and F1.2. The amplicons obtained by RT-PCR were directly sequenced.

The composite of the human SCA2 cDNA sequence assembled from several overlapping cDNA clones is shown in Figure 6 (SEQ ID NO:2). The longest open reading frame consists of 3936 bp and ends with a TAA termination codon. The stop codon is followed by 364 bp of 3' untranslated sequence. The CAG repeat is located in the 5' end of the coding region. The putative translation start site follows an in frame stop codon located 78 bp upstream. The predicted molecular weight for the SCA2 translation product is 140.1 kDa with the CAG trinucleotide repeat predicted to code for glutamine. In analogy to the SCA1 gene product, we propose the name ataxin-2 for the SCA2 gene product.

The cDNA sequence was compared against the GenBank database using the FASTA sequence alignment algorithms and the TIGR database. The predicted protein sequence was compared against the SwissProt database and the predicted translation products of the GenBank database. These searches revealed no significant

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similarities to genes of known function except for limited homologies to the GLI-Krueppel related protein YY1 (nucleotides 45 to 586, odds against chance occurrence  $6.6 \times 10^{-7}$ ).

5

However, significant similarities were detected with two partial cDNA transcripts in the TIGR database (THC148678, H03566, odds against chance similarity  $<10^{-31}$ ). Complete sequence analysis of these cDNA clones  
 10 (purchased from ATCC) revealed significant homologies with ataxin-2. This protein was named ataxin-2 related protein (A2RP). The region showing the most significant homology including a domain of 42 amino acids with 86% identity (codons 243-284 of the consensus sequence) is  
 15 shown in Figure 7. This domain is also 100% conserved in mouse ataxin-2. Despite the significant homologies, the polyglutamine tract in ataxin-2 was replaced with an interrupted polyproline tract in the related A2RP human protein and was reduced to one glutamine in the mouse  
 20 SCA2 homologue (see Figure 7).

#### Example 6

##### RT-PCR and Northern blot analysis:

25 RNA isolation and reverse transcription was carried out using well-known methods (Huynh et al., 1994, Hum. Mol. Genet., 3:1075-1079). RNAs were isolated from lymphoblastoid cell lines established from patients and unrelated spouses in the FS pedigree with SCA2 (Pulst et  
 30 al., 1993, Nat. Genet., 5:8-10). Multiple tissue Northern blots were purchased from Clontech. For amplification, primers located in two exons (SCA-A and SCA-B14, see also Figure 6) were chosen so that genomic DNA was not amplified. The sequence for SCA-B14 was:  
 35 5' TTCTCATGTGCGGCATCAAG.

(GEL IS NO. 13)

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Using RT-PCR, it was determined that the SCA2 CAG repeat was transcribed in lymphoblastoid cell lines. In cDNAs from SCA2 patients, transcription from both the normal and the expanded allele was detected using  
5 oligonucleotide primers that flank the repeat. By Northern blot analysis, the SCA2 gene was determined to be widely expressed. A strong signal corresponding to a 4.5 kb transcript was detected in all brain regions examined. This transcript was also detected in RNAs  
10 isolated from heart, placenta, liver, skeletal muscle, and pancreas. Little transcript was detected in lung and no transcription was detectable in kidney. A much fainter transcript of 7.5 kb could be seen in RNAs isolated from some brain regions and in some peripheral  
15 tissues.

#### EXAMPLE 7

##### Isolation of mouse SCA2 cDNA

20 To identify mouse SCA2 cDNA clones, the Stratagene Lambda ZAP newborn mouse brain cDNA library was screened with a human SCA2 cDNA clone. Six clones were identified and sequenced. A full-length mouse SCA2 cDNA is set forth in SEQ ID NO:4.

25

##### SUMMARY OF SEQUENCES

SEQ ID NO:1 is the genomic nucleic acid sequence set forth in Figure 2.

30

SEQ ID NO:2 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a human-derived SCA2 protein of the present invention (also set forth in Figure 6).

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B6T50" B66T B660

SEQ ID NO:3 is the deduced amino acid sequence of the human-derived SCA2 protein set forth in SEQ ID NO:2.

5           SEQ ID NO:4 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a mouse-derived SCA2 protein of the present invention.

10           SEQ ID NO:5 is the deduced amino acid sequence of the mouse-derived SCA2 protein set forth in SEQ ID NO:4.

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